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Comparison between the photocatalytic inactivation of Gram-positive *E. faecalis* and Gram-negative *E. coli* faecal contamination indicator microorganisms

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ABSTRACT

Photocatalytic inactivation of two different faecal contamination indicator microorganisms, the Gram-negative *Escherichia coli* and the Gram-positive *Enterococcus faecalis*, has been studied using TiO_2 in suspension and immobilized onto the reactor wall. The effect of the main variables of the photocatalytic process on the disinfection efficiency has been analyzed using deionized water and a simulated effluent of wastewater treatment plant (WTP). Noticeable differences were observed between both types of bacteria during photolytic experiments without TiO_2 in deionized water, probably due to the higher sensibility of *E. coli* to the osmotic stress, which leads to a higher cell membrane permeability and consequently a lower amount of hydroxyl radical attacks required to overcome the inactivation threshold. In contrast, despite their structural differences, Gram-positive and Gram-negative bacteria seem to follow the same inactivation mechanism, showing no significant differences in the experiments carried out with TiO_2 in suspension either in deionized water or in WTP simulated effluent, with similar responses to changes in the concentration of catalysts and irradiation power (both variables involved in the hydroxyl radical generation). Similar results are observed using immobilized TiO_2 in deionized water, although disinfection experiments of WTP simulated effluent using immobilized TiO_2 showed much longer initial delays before the beginning of the inactivation for *E. faecalis*, suggesting a critical effect of the water composition of the bacteria-catalyst interaction. In any case, the irradiation time required to achieved the inactivation below the experimental bacterial detection limit is similar for both microorganisms, and experiments with mixtures of *E. faecalis* and *E. coli* in WTP simulated effluent show no significant differences. Therefore, it can be concluded that the results of photocatalytic disinfection experiments using *E. coli* as model bacteria could be reasonably extrapolated to other types of bacteria or bacterial mixtures.

1. Introduction

Water disinfection is still a scientific and technical challenge since conventional methods used to supply drinking water such as chlorination and ozonation have shown disadvantages related to the formation of potentially hazardous disinfection by products (DBPs), resulting from the reaction of the oxidant species with the natural organic matter present in the water [1].

Advanced Oxidation Technologies (AOT) have been investigated with the aim of developing new disinfection methods to supply drinking water. Among them, heterogeneous photocatalysis is considered a promising technology. It is based on the interaction between light and solid semiconductor particles to produce highly oxidising hydroxyl radicals (•OH). Main efforts have been focused on TiO_2 photocatalysis as the catalyst is inexpensive, non-toxic and stable, allowing the operation under ambient conditions of pressure and temperature and without addition of chemicals different from the air [2]. Moreover, its low energy consumption and the possibility of using solar energy will be crucial for developing countries where about 90% of infectious diseases are attributed to unsafe water supply, inadequate sanitation and hygiene [3].

Several studies have reported the feasibility of photocatalytic processes for both organic matter degradation and bacteria inactivation [4,5]. However, very few studies have reported the photocatalytic inactivation of microorganisms in real waters [6–9] and most of them have only used one model bacteria group. Considering that some differences in photocatalytic inactivation response have been reported for different type of microorganisms and waters with different chemical composition [10], more efforts should be focused on the establishment of possible differences in the photocatalytic disinfection efficiency depending on the selected indicator microorganism.

The most commonly accepted photocatalytic inactivation mechanism is based on the attack of •OH radicals and other reac-

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tive oxygen species (ROS) to the bacteria cell wall, where the bacteria-catalyst contact takes place [11,12]. Cumulative damage leads to the cell membrane disorder, increasing its permeability, demonstrated by the leakage of potassium ions [13,14], and finally the cell lysis and death. Therefore, since the attack occurs on the bacteria outer wall, more attention must be paid to the differences of wall structure which may also lead to different photocatalytic inactivation efficiency. In this sense, the differences between Gram-negative and Gram-positive bacteria, two groups of prokaryotic microorganisms, could be considered as example. Gram-negative microorganisms have a thinner peptidoglycane cell wall than Gram-positive, but they have an additional outer membrane containing two lipid bilayers which provide them a higher complexity [15,16].

The aim of this work is to compare the efficiency of the photocatalytic inactivation of the Gram-negative *Escherichia coli* and the Gram-positive *Enterococcus faecalis*, two bacterial species commonly used as faecal contamination indicator microorganisms with a different cell structure. The investigation has been focused on the influence of the main variables of the process, such as catalyst concentration, irradiation power and initial bacterial concentration, together with the use of two different ways of using TiO_2 catalyst, as slurry and immobilized on the reactor wall.

2. Experimental

Photocatalytic experiments have been carried out by means of an experimental setup consisting of an annular photoreactor 15 cm long, 3 cm inner diameter and 5 cm external diameter operating in a closed recirculating circuit with a stirred reservoir tank for a total working volume of 1 L. Two different reactor configurations were used. A slurry reactor, using Degussa P25 titanium dioxide powder in suspension, and a wall reactor, using the same catalyst immobilized onto the 15 cm long glass tube that constitutes the inner wall of the reactor. If not otherwise specified, a concentration of 0.1 g L^{-1} and two TiO_2 coating cycles were used for the TiO_2 suspension and the immobilized system, respectively, being both values optimized in previous studies [9]. Illumination was performed using a Philips TL 6W black light lamp placed in the axis of the reactor with an emission maximum at 365 nm. The UVA incident photon flow, determined by ferrioxalate actinometry, was 2.8×10^{-6} Einstein s^{-1}. The irradiation power entering the reactor was controlled by using neutral filters placed in the light path between the lamp and the inner reactor wall.

Two different strains of bacteria, *E. coli* K12 (ATCC 23631) and *E. faecalis* (ATCC 11700), provided by the CECT (Colección Española de Cultivos Tipo) were used for the inactivation experiments. *E. coli* was grown in Luria Bertani Broth (Miller's LB Broth, Scharlab) and LB nutrient agar (Miller's LB Agar, Scharlab) as liquid and solid culture media whereas Tryptic Soy Broth (TSB, Scharlab) and Tryptic Soy Agar (TSA, Scharlab) were used for *E. faecalis*.

Fresh liquid cultures with stationary concentrations around 10^9 CFU mL^{-1} were prepared collecting 100 μL of a mother culture in the stationary growing phase corresponding to *E. coli* or *E. faecalis* and inoculating them in 20 mL of fresh growing medium. Then, they were incubated at 37 °C for 24 h on a rotary shaker. The suspensions for reaction, of about 10^6 CFU mL^{-1} if not otherwise specified, were prepared by centrifuging 5 mL of the liquid culture at 3000 rpm for 15 minutes. After resuspension with 5 mL sterile deionized water (Milli-Q®, 18.2 MΩ cm), 1 mL of the suspension was added to water and made up to 1 L. For the experiments carried out with mixtures of *E. coli* and *E. faecalis*, the initial concentration of the total bacteria suspension was also of about 10^6 CFU mL^{-1}.

Two aqueous media were used: (i) deionized water (Milli-Q®, 18.2 MΩ cm), and (ii) synthetic municipal wastewater [17] diluted to an average total organic carbon value of 15 mg L^{-1} to simulate wastewater treatment plant (WTP) effluents. The concentration of viable bacteria along the reaction was followed through a standard serial dilution procedure. Each decimal dilution was spotted eight times on nutrient agar plates and incubated at 37 °C for 24 h before counting. LB nutrient agar was used to follow the total inactivation. To follow *E. coli* and *E. faecalis* inactivation individually two selective agars were used, Colinstant Chromogenic Agar and Slanetz & Bartley Agar (Scharlab), respectively.

3. Results and discussion

3.1. Non-photocatalytic reference experiments

Before studying the photocatalytic inactivation of both types of bacteria, preliminary experiments of non-photocatalytic inactivation under different stressful conditions were carried out. Fig. 1 shows the effect of UVA light irradiation, stirring and presence of TiO_2 particles in suspension (in dark conditions) on the decrease in the concentration of *E. coli* and *E. faecalis* viable bacteria both in deionized water and in WTP effluents. All the experiments performed in WTP effluents show null inactivation, including those carried out in the dark with and without stirring (not shown).

No significant inactivation is observed for both types of bacteria suspended in deionized water in the dark, even after a week (not shown in the figure). However, when the system is stirred a significant decrease in the amount of viable *E. coli* bacteria is observed, whereas the effect is negligible on *E. faecalis*. Therefore, *E. coli* seems to be very sensitive (more than *E faecalis*) to mechanical damages derived from the stirring, as also observed by Sichel et al. [18].

The presence of TiO_2 particles in the stirred suspension using deionized water without irradiation increases even more the inactivation rate of *E. coli*, whereas the effect on *E. faecalis* can be also neglected. In contrast, the inactivation of *E. coli* in WTP simulated effluent is negligible even in the presence TiO_2 particles, being in this case the results comparable to those of *E. faecalis*.

UVA light irradiation in deionized water leads to significant differences between both microorganisms, since *E. coli* shows a 5 log-decrease of the concentration of viable bacteria after 6 h of phototreatment whereas for *E. faecalis*, only a 3 log-decrease is observed, with a longer initial delay. In contrast, no inactivation has been observed for both kinds of bacteria during the same UVA light irradiation time in WTP simulated effluent. The inactivation response showed by both *E. coli* and *E. faecalis* in deionized water is probably due to the DNA damage to the bacteria caused by the reactive oxygen species (ROS) produced by UVA radiation [19]. Nevertheless, the lack of inactivation in WTP simulated effluent cannot be explained by competence between bacteria and ions and organic matter for the ROS, as they are formed within the cell. Moreover, absorption of photons by anions and organic matter can be dismissed since it does not occur under the studied wavelength range. Consequently, these results could be due to the fact that the chemical species present in water might serve as nutrients for bacteria [7] or more probably to an osmotic effect.

The different UVA inactivation behaviour observed between *E. coli* and *E. faecalis* is still unclear since opposite explanations have been suggested by different authors. For instance, Robertson et al. [19] and Chung et al. [20] suggested a possible different inactivation mechanism response to UVA light for Gram-negative and Gram-positive due to possible differences in the protection, inactivation and repairing mechanisms against light. In contrast, Rincón and Pulgarín [21] explained these differences focusing on a thicker cell wall presents in *E. faecalis* which provides more resistance to the ROS attacks. In addition to that, the osmotic stress caused by deionized water has been reported by several authors [10,18] as a factor

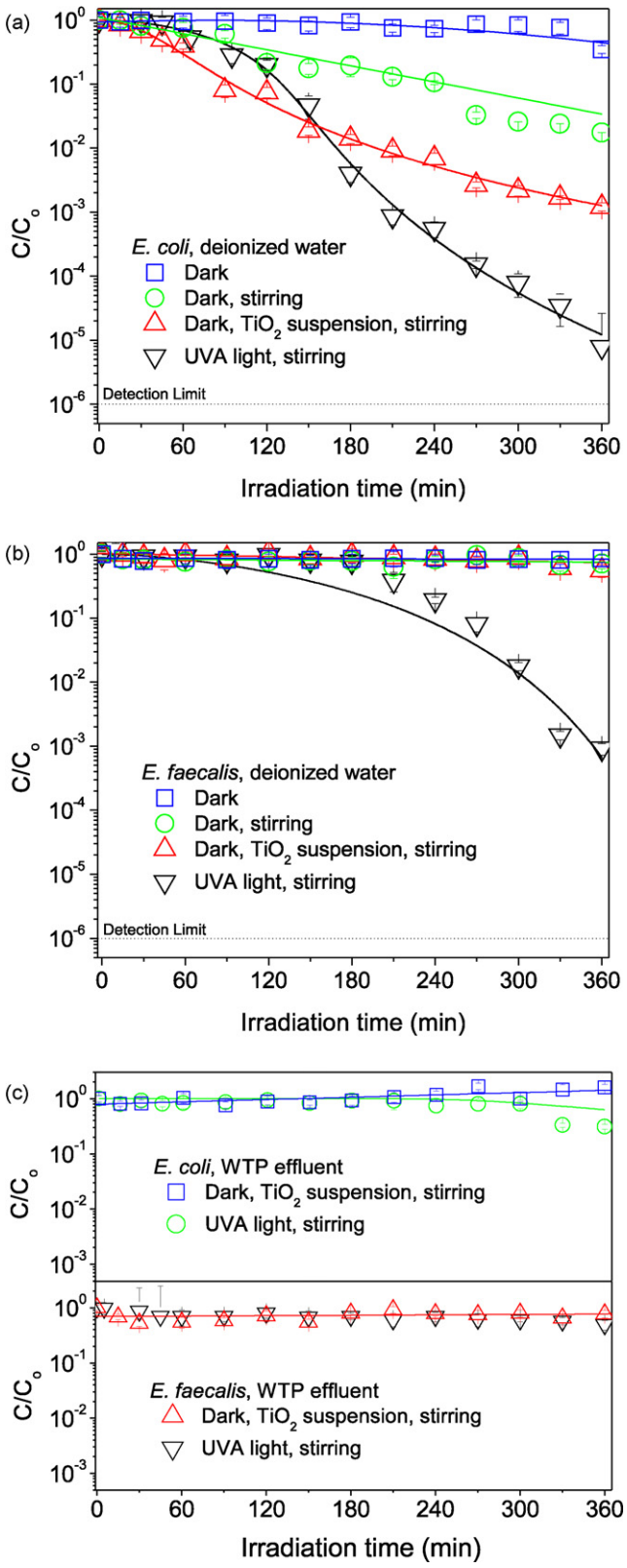

Fig. 1. Non-photocatalytic inactivation of *E. coli* and *E. faecalis* under different stressful conditions in deionized water and WTP simulated effluent. Fitting lines were calculated using a kinetic model based on a series event disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements.

to increase the cell membrane permeability favoring the leakage of ions from the cell and the subsequent cell death. Therefore, the higher *E. coli* inactivation in deionized water suggests that *E. coli* seems to be more sensitive to the osmotic stress than *E. faecalis*, requiring fewer ROS attacks to produce cell lysis. *E. faecalis* has a thicker cell wall, probably the responsible of a lower sensitivity by osmotic stress, which together with the ability to form a protein capsule under stressful conditions, increase the bacteria protection [21]. This stronger osmotic stress on *E. coli* is confirmed by the experiments carried out in WTP simulated effluent in which no inactivation is observed for both types of bacteria due to the presence of ions and organic matter. Additionally, experiments carried out under stirring in deionized water, in which only *E. coli* inactivation was observed, suggest that the weakening of the bacterial cell wall makes *E. coli* more sensitive that *E. faecalis* to the inactivation due to the mechanical stress given by the stirring when cells are simultaneously under osmotic stress.

Concerning the presence of titanium dioxide particles in the suspension, despite TiO_2 might possess properties to affect the bacterial viability even in the dark [22], the opposite behaviour observed in experiments with *E. coli* in deionized water and in WTP simulated effluents, suggesting that the osmotic stress should be considered again. Although the outer membrane of Gram-negative bacteria have been reported to limit the permeability to many chemical compounds [23,24], the osmotic stress might change the permeability of the cell wall, allowing the transfer of the smallest TiO_2 particles through the cell wall, as suggested by the results of Huang et al. [14]. This would be in agreement with the higher inactivation rate observed for *E. coli*, more sensitive to osmotic stress than *E. faecalis* due to its thinner cell wall, and with the absence of inactivation of both Gram-negative and Gram-positive bacteria in WTP simulated effluent where there are no conditions of osmotic stress.

To sum up, these reference experiments show that *E. coli* is more sensitive compared to *E. faecalis* under osmotic stress in deionized water together with additional stressful conditions such as UVA irradiation, stirring or presence of TiO_2 particles in suspension. In contrast, in WTP simulated effluent, without osmotic stress, both *E. coli* and *E. faecalis* seems to show a much higher resistance against mechanical and radiative stressful factors. Consequently, it seems that the activity of the osmoregulatory system of *E. coli* inhibits the action of the protection, inactivation and repairing mechanisms against other stressful factors such as UVA light or mechanical damage.

3.2. Photocatalytic experiments with TiO_2 in suspension

Fig. 2 shows the comparison of the photocatalytic inactivation of *E. coli* and *E. faecalis* in deionized and WTP effluents using 0.1 g L^{-1} of TiO_2 in suspension. Compared to the reference experiments described in the previous section, the photocatalytic process leads to a more effective inactivation, since a higher number of hydroxyl radicals and other ROS are produced. Both types of microorganisms are successfully inactivated, achieving a 6-log-decrease of the concentration of viable bacteria and reaching a value below the detection limit after 2 h and 4 h of irradiation in deionized water and WTP simulated effluent, respectively. These results confirm the strong influence of water composition on the efficiency of the photocatalytic process since ions and organic matter present in water compete with bacteria for hydroxyl radicals and also hinder the interaction bacteria-catalyst [25,26].

Despite the results of the reference experiments, no differences in the sensibility of *E. coli* and *E. faecalis* to the photocatalytic process are observed, neither in deionized water nor in WTP simulated effluent, since inactivation profiles are quite similar and the reaction time required to achieve the bacterial detection limit is similar

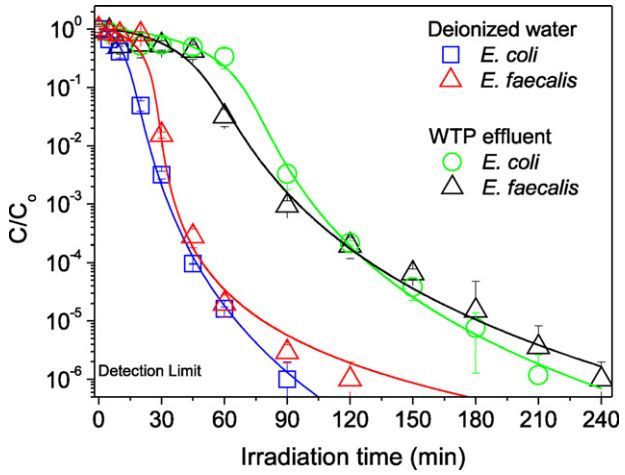

Fig. 2. Photocatalytic inactivation of *E. coli* and *E. faecalis* with TiO_2 in suspension in deionized water and WTP simulated effluent (TiO_2 concentration: 0.1 g L^{-1}; irradiation power: 2.8×10^{-6} Einstein s^{-1}; initial concentration of bacteria: 10^6 CFU mL^{-1}). Fitting lines were calculated using a kinetic model based on a series events disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements.

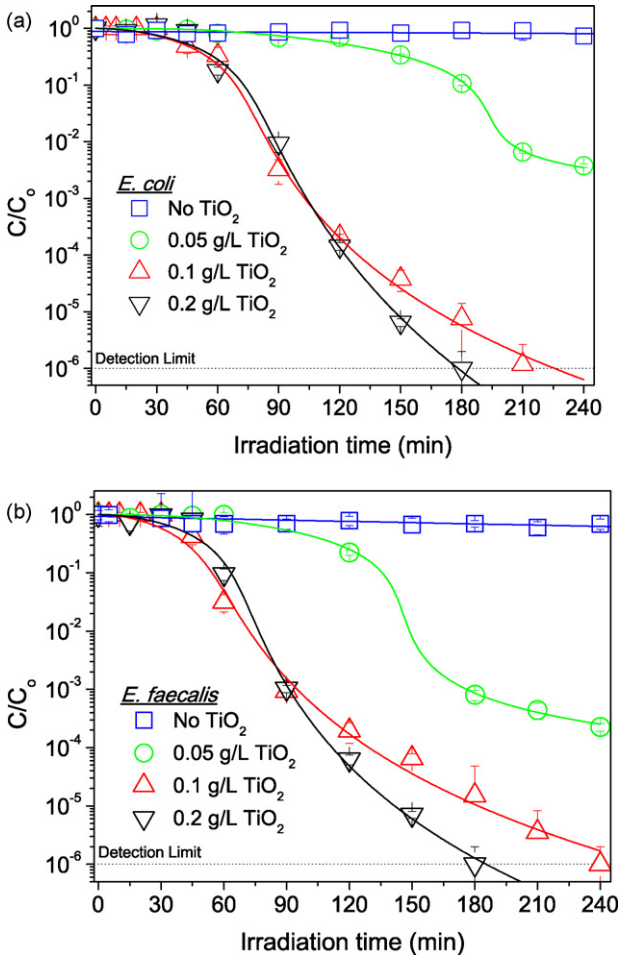

Fig. 3. Photocatalytic inactivation of *E. coli* and *E. faecalis* with increasing concentration of TiO_2 in suspension in WTP simulated effluent (irradiation power: 2.8×10^{-6} Einstein s^{-1}; initial concentration of bacteria: 10^6 CFU mL^{-1}). Fitting lines were calculated using a kinetic model based on a series event disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements.

in both cases, in agreement with previous reports [6,27,28]. Consequently, •OH attack to the cell wall seems to be quite effective for both, Gram-negative and Gram-positive photocatalytic inactivation, being negligible the higher sensibility to mechano-osmotic stress observed for *E. coli*.

Hydroxyl radical attack on the cell wall is probably the first step which leads to the bacterial lysis, as it has been reported by several authors for *E. coli* inactivation [12,14,23,28–30] and also for other types of microorganisms [20,31–36]. However, several authors have suggested different inactivation mechanisms for Gram-negative and Gram-positive bacteria [16,20,37] and some of them even have pointed out different photocatalytic response, showing a strong contradiction [16,35].

Gram-positive has been reported to be photocatalytically more resistant than Gram-negative due to a thicker cell wall. As a result, a higher number of hydroxyl radical attacks for Gram-positive bacteria are needed to get the complete bacterial inactivation [11,20,31,38,39]. Chung et al. [20] and Shaomin et al. [36] reported similar photocatalytic inactivation mechanism for Gram-positive and Gram-negative bacteria, but the thinner wall of *E. coli* provided less protection against oxidant species. In addition to this, several authors have reported a higher adhesion of *E. coli* onto immobilized TiO_2, which favors the interaction bacteria-catalyst and consequently the inactivation [40–42].

In contrast, Gram-negative has been also suggested being more resistant to the photocatalytic process due to a more complex structure given by the additional outer membrane which might protect it towards disinfectant agents [23,24,33,43]. Although the thicker cell wall of Gram-positive bacteria is hardly broken by hydroxyl radicals, the lack of this outer membrane makes easier for the hydroxyl radicals to damage the bacterial DNA. Although Skorb et al. [35] found Gram-negative cells more sensitive, they also highlighted the possible influence of the chemical composition of the cell wall, which might lead to a different resistance to •OH, hydrophobicity or electrostatic charge. Demidova and Hamblin [44] also explained the higher resistance showed by Gram-negative against Gram-positive due to the differences in chemical composition of the cell wall and protection mechanisms. Finally, Sinton et al. [45] supported the different results observed for both kinds of microorganisms as a function of water composition together with different defense mechanisms.

In order to shed light on this controversy, we have carried out further experiments in different experimental conditions of catalyst concentration, irradiation power, initial bacterial concentration, using also an immobilized TiO_2 system for which presumably a different bacteria-catalyst interaction takes place.

3.2.1. *Effect of TiO_2 concentration*

Experiments carried out using different concentrations of titania in deionized water in the range from 0.02 to 0.5 g L^{-1} showed similar trends for both microorganisms, with an improvement in the efficiency of the process as the TiO_2 concentration increases, reaching a plateau for 0.1–0.2 g L^{-1}, in agreement with previous results of *E. coli* inactivation in this reactor [46]. Similar trends have been reported for *E. coli* by other authors [7,47–50] what has been obviously attributed to the increase in the amount of photons that can be absorbed for higher amounts of titania before reaching a maximum corresponding to the total absorption of the incident radiation.

Concerning experiments in WTP simulated effluent, Fig. 3 shows the comparison of the inactivation of both types of bacteria for increasing concentrations of titania. Although the irradiation time required for the total inactivation is obviously higher in comparison with experiments in deionized water, once again, similar trends are observed for both microorganisms, showing the maximum inactivation efficiency for values similar values around 0.1–0.2 g L^{-1} of TiO_2.

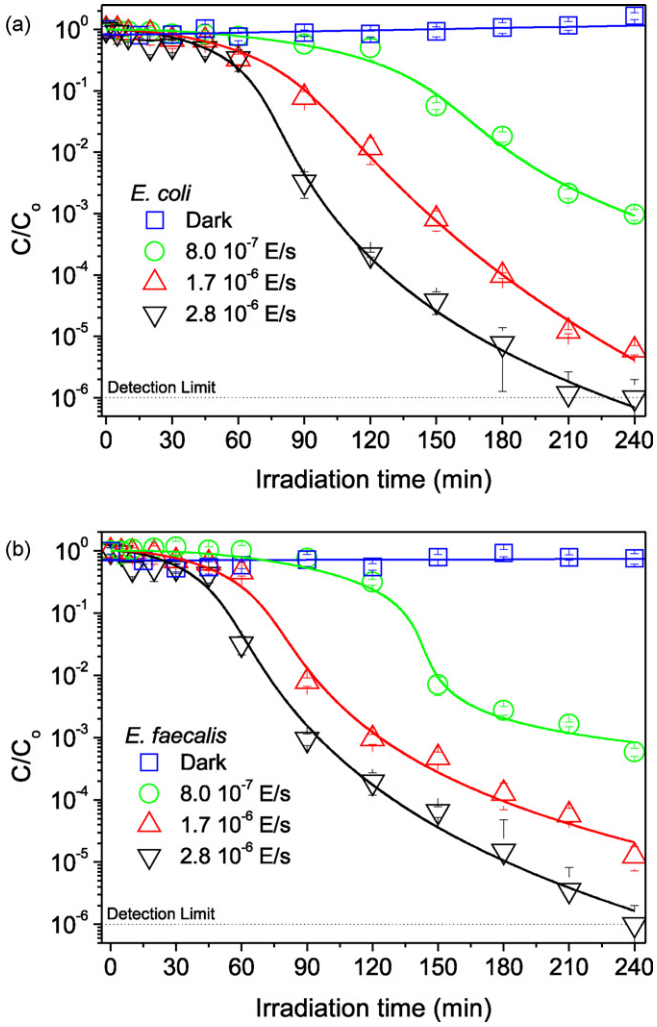

Fig. 4. Photocatalytic inactivation of *E. coli* and *E. faecalis* with TiO_2 in suspension using increasing irradiation power in WTP simulated effluent (TiO_2 concentration: 0.1 g L^{-1}; initial concentration of bacteria: 10^6 CFU mL^{-1}). Fitting lines were calculated using a kinetic model based on a series event disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements.

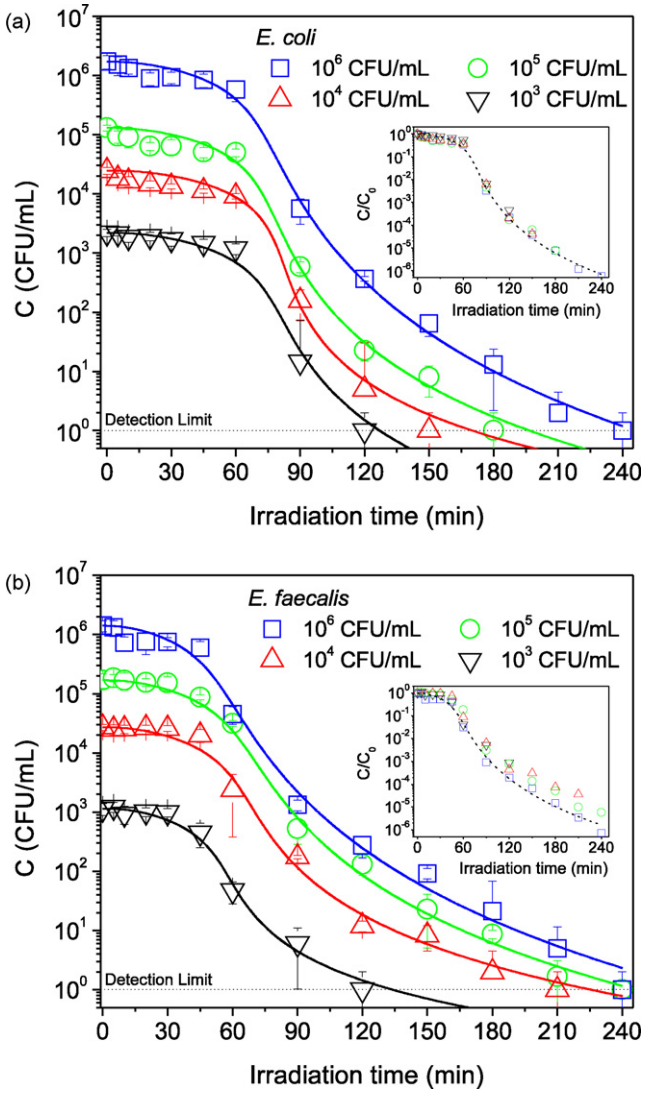

Fig. 5. Photocatalytic inactivation of WTP simulated effluent with increasing initial concentrations of *E. coli* and *E. faecalis* using TiO_2 in suspension (TiO_2 concentration: 0.1 g L^{-1}; irradiation power: 2.8×10^{-6} Einstein s^{-1}). Fitting lines were calculated using a kinetic model based on a series event disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements. Inset plots represent the results of all the experiments in relative terms as C/C_0.

Summarizing, no significant differences have been observed between the photocatalytic inactivation of Gram-negative and Gram-positive bacteria for all experiments and the optimal concentration of TiO_2 for this photoreactor seems to be the same independently on the type of bacteria or the type of water. Consequently, it can be concluded that despite their differences in cell wall structure both *E. coli* and *E. faecalis* show similar interaction with the catalyst, being the influence of this variable essentially related to the absorption of radiation.

3.2.2. Effect of irradiation power

Fig. 4 depicts the effect of the incident irradiation power over the photocatalytic inactivation of *E. coli* and *E. faecalis* in WTP simulated effluent. A clear increase in the efficiency of the process is observed for higher irradiation power, being this trend very similar for both types of microorganisms, and similar for experiments carried out with deionized water (not shown) in agreement with results for *E. coli* previously reported [46].

This faster inactivation of *E. coli* for increasing irradiation power has been also reported for other research groups [7,47–53] and is obviously related to an increase in the hydroxyl radical production. This relation seems to be close to the linear dependence between photocatalytic inactivation and •OH radical concentration found by Cho et al. [12], what suggests that the range of incident irradiation power used in these experiments is in the low-intensity linear region corresponding to the optimal light utilization [2,54].

The most important conclusion of this section is that no significant differences have been observed between the photocatalytic inactivation of Gram-negative and Gram-positive bacteria. Consequently, it can be concluded that irradiation power and catalyst concentration are responsible for the rate of radiation absorption and hydroxyl radical generation, being negligible the differences in the interaction with each type of bacteria. Moreover, for equivalent rates of hydroxyl radicals generation, the inactivation of both microorganism is very similar, leading to the conclusion that the inactivation mechanism should be quite similar, despite the differences in the cell wall structure.

3.2.3. Effect of the initial concentration of bacteria

Both microorganisms are successfully inactivated below the detection limit for all the tested initial concentrations, requiring shorter irradiation times the experiments in deionized water (not shown) than those in WTP simulated effluent (Fig. 5), as it was

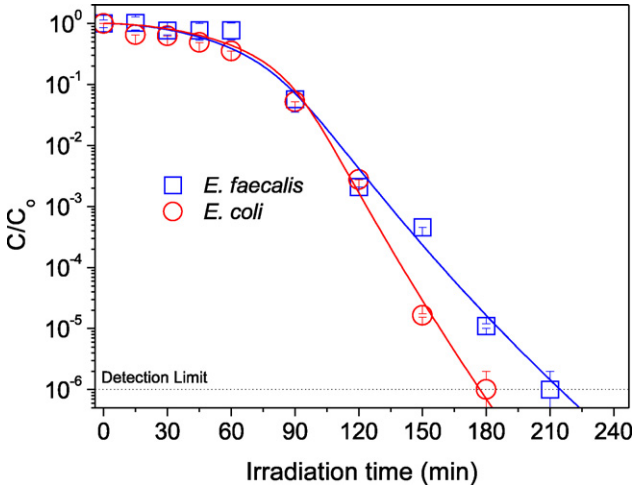

Fig. 6. Photocatalytic inactivation of a 50/50 mixture of *E. coli* and *E. faecalis* in a WTP simulated effluent using TiO_2 in suspension (TiO_2 concentration: 0.1 g L^{-1}; irradiation power: 2.8×10^{-6} Einstein s^{-1}; initial concentration of bacteria: 10^6 CFU mL^{-1}). Fitting lines were calculated using a kinetic model based on a series event disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements.

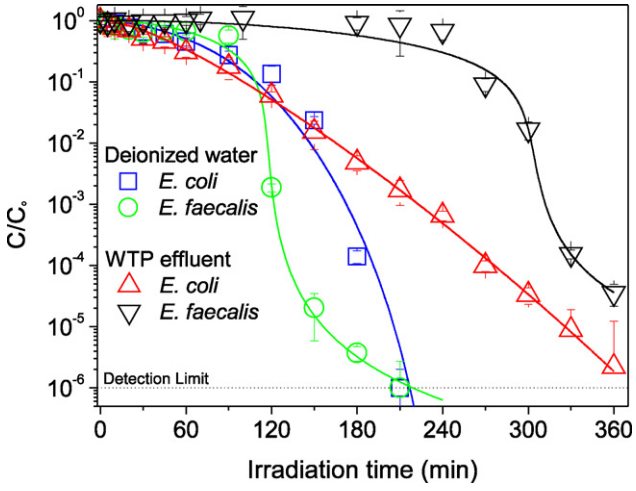

Fig. 7. Photocatalytic inactivation of *E. coli* and *E. faecalis* with immobilized TiO_2 in deionized water and WTP simulated effluent (irradiation power: 2.8×10^{-6} Einstein s^{-1}; initial concentration of bacteria: 10^6 CFU mL^{-1}). Fitting lines were calculated using a kinetic model based on a series event disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements.

expected. The trend was very similar in all cases, in contrast with the results reported by Kerr et al. [55], who pointed out the different influence of water quality on the inactivation for different initial concentrations.

The increase in the irradiation time required for the total inactivation for higher bacterial concentrations is in agreement with results provided by other authors [21,22,39,42,50,51,56]. They also reported an increase in the kinetic constant, *k*, as the initial concentration is higher. However, our results lead to very similar values of *k* (the slope of the inactivation curves), following all the experiments the same kinetics in terms of C/C_0 (see inset of Fig. 5), what means that the time required for a complete inactivation is not as significant as the increase in initial bacterial concentration. Therefore, these results are in agreement with the independence of *k* for initial concentrations between 10^3 and 10^{10} CFU mL^{-1}, as it was reported by Fernández-Ibáñez [57].

It can be concluded that both microorganisms are inactivated through a similar sequence of first-order processes with a kinetic constant dependent on the catalyst concentration and the irradiation power but with a reaction rate limited by the concentration of bacteria.

3.2.4. Mixtures of E. coli and E. faecalis

Finally experiments of photocatalytic inactivation of mixtures with similar concentrations of *E. coli* and *E. faecalis* in WTP simulated effluent were carried out, being the results depicted in Fig. 6. Although the curves suggest that *E. coli* seems to be slightly more sensitive to the treatment, specially for long irradiation times, these differences can not be considered as significant taking into account that both microorganisms have been quantified in selective culture media with the limitation of the procedure to distinguish selectively the growth of each type of bacteria.

In conclusion, although several differences have been reported between Gram-negative and Gram-positive bacteria in the photocatalytic inactivation, mostly based in the different structure and chemical composition of the cell wall, electrostatic charge, and adhesion properties that influence the interaction bacteria-catalyst, our results suggest that these differences do not seem to be crucial for the efficiency of the process, neither in deionized water nor in WTP simulated effluent. Therefore, the results obtained in the photocatalytic experiments with TiO_2 in suspension using *E. coli* as model bacteria could be reasonably extrapolated to other bacteria families, not only to other Gram-negative species with reported higher resistance to UVA [58], but also to Gram-positive bacteria with significant structural differences in the cell wall.

3.3. Photocatalytic experiments with immobilized TiO_2

For the following experiments the photoreactor geometry and radiation source are exactly the same as the one used previously, except the substitution of the inner glass reactor wall for a equivalent TiO_2-coated glass wall. Therefore, photolytic experiments showed in Fig. 1 are also valid as reference for the immobilized systems. Bacterial inactivation with immobilized TiO_2 commonly requires longer irradiation times in comparison with TiO_2 in suspension, as it is confirmed in Fig. 7 for both types of microorganisms and waters. This effect is usually attributed to the lower TiO_2 surface available for the interaction with bacteria [7,9,11,21,50]. Nevertheless, the increase in the duration of the treatment to reach bacterial concentrations below the detection limit can be counteracted by the simplification of the process derived from avoiding the separation step of the catalyst in the global process, making immobilized systems more interesting for real applications in continuous processes. For that reason, possible differences in the efficiency of the photocatalytic inactivation of *E. coli* and *E. faecalis* have been also investigated for this reactor configuration.

No significant differences between the irradiation time required to reach the bacterial detection limit with *E. coli* and *E. faecalis* in deionized water are observed. Inactivation of both bacteria in WTP simulated effluent also requires similar irradiation times for both microorganism, much longer than that required in experiments with deionized water. However, it must be noticed that the shape of the curves is quite different, showing *E. faecalis* experiments much longer initial delays and faster inactivation rates once the concentration in viable bacteria starts to decrease, whereas in *E. coli* curves the inactivation begins almost from the beginning of the experiment but with a slower inactivation rate. This behaviour is observed in both types of water, but not in experiments with TiO_2 in suspensions, what suggests that the reason behind such behaviour should be found on the different interaction between each type of bacteria and the immobilized TiO_2 catalyst.

Fu et al. [23] and Page et al. [24] support that since *E. faecalis* cell wall is thicker than that of *E. coli*, it needs more hydroxyl radical attacks to be broken. However, when the cell wall is damaged, it is

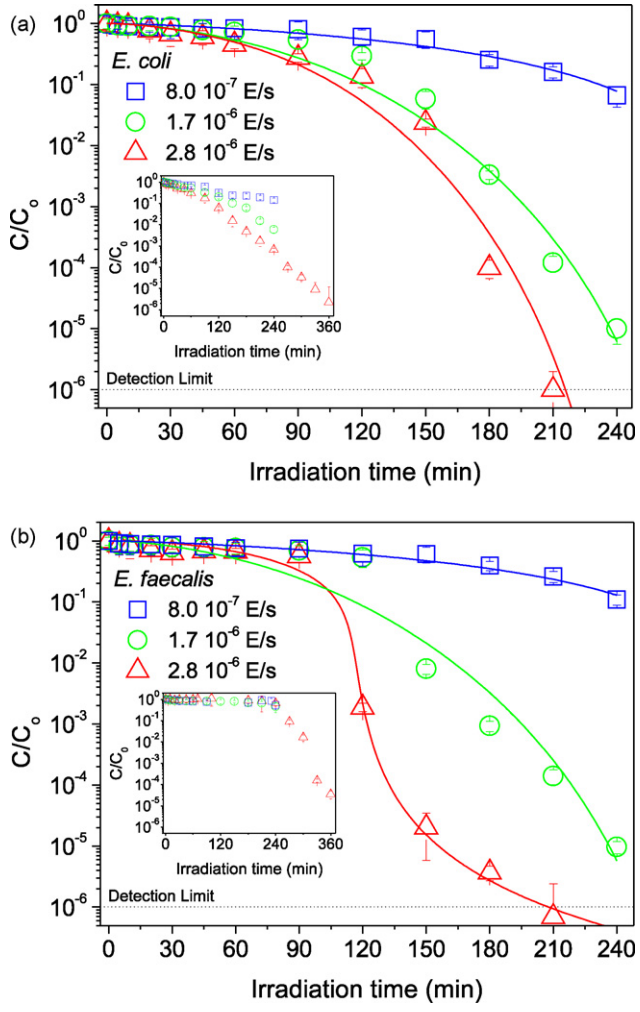

Fig. 8. Photocatalytic inactivation of *E. coli* and *E. faecalis* with immobilized TiO_2 using increasing irradiation in deionized water (figures) and WTP simulated effluent (inset) (TiO_2 concentration: 0.1 g L^{-1}; initial concentration of bacteria: 10^6 CFU mL^{-1}). Fitting lines were calculated using a kinetic model based on a series event disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements.

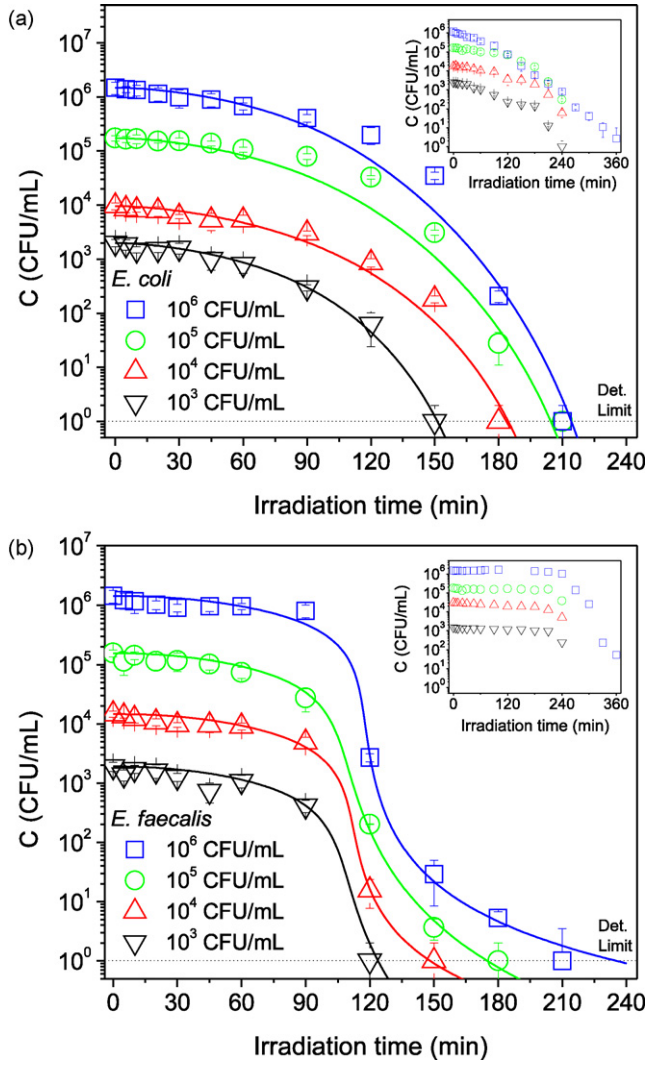

Fig. 9. Photocatalytic inactivation of deionized water (figure) and WTP simulated effluent (inset) with increasing initial concentrations of *E. coli* and *E. faecalis* using immobilized TiO_2 (TiO_2 concentration: 0.1 g L^{-1}; irradiation power: 2.8×10^{-6} Einstein s^{-1}). Fitting lines were calculated using a kinetic model based on a series event disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements.

easier for the OH radicals to reach the cytoplasmic membrane since *E. faecalis* has a less complex cell wall morphological structure than *E. coli*. Because of that, a longer initial delay is observed for the *E. faecalis* kinetic profile, but the detection limit is achieved at comparable irradiation times than *E. coli*. However, this explanation is not in agreement with the results observed using TiO_2 suspensions, in which no significant differences between the shapes of the curves are found. Bui et al. [56] reported that the surface area, particle size and surface charge modify the bacteria-TiO_2 interaction since different inactivation activities were observed when different kinds of TiO_2 in suspension were used. These differences in bacteria-TiO_2 adhesion were also reported by Cohen-Yaniv et al. [42] who observed differences in the photocatalytic activity for different Gram-negative bacteria using slurry and immobilized catalysts. The photocatalytic activity was higher for the most hydrophobic bacteria tested when immobilized catalyst was used. Due to the higher hidrophylicity of *E. coli*, other Gram-negative bacteria, more hydrophobic than *E. coli* showed more adherence and interaction with the immobilized catalyst, while no differences in efficiency were observed when TiO_2 was used in suspension. Manjón et al. [27] also observed higher photocatalytic activity for *E. faecalis* than for *E. coli* using a hydrophobic ruthenium (II) catalyst, whereas Li and Logan [40] also reported a higher Gram-negative – TiO_2 adhesion than Gram-positive – TiO_2 adhesion. Accordingly, these differences might be more important when the catalyst is immobilized, since the surface available for bacteria-TiO_2 interaction decreases. From all this it seems that the chemical composition of the microorganisms cell wall leads to differences in hydrophobicity which might significantly affect the cell-catalyst adhesion, as it is also shown by Sichel et al. [59] for other types of non-bacterial microorganisms.

These differences in the bacteria-TiO_2 interaction are not so pronounced in experiments in deionized water, what means that the chemical composition of the suspension must be also playing a role. The presence of chemicals in the water remove the bacterial osmotic stress existing in deionized water and compite for the hydroxyl radicals responsible for the inactivation, but also influences the bacteria-catalyst adhesion. In fact, Demidova and Hamblin [44] reported that the compounds present in the aqueous suspension could get into some bacteria more easily than to others due to their differences in charge, chemical composition and cell structure, and could even modify the bacterial electrostatic charge, leading to changes in the bacteria-catalyst interaction [25,59]. Moreover, at slightly alkaline pH values (typical for WTP

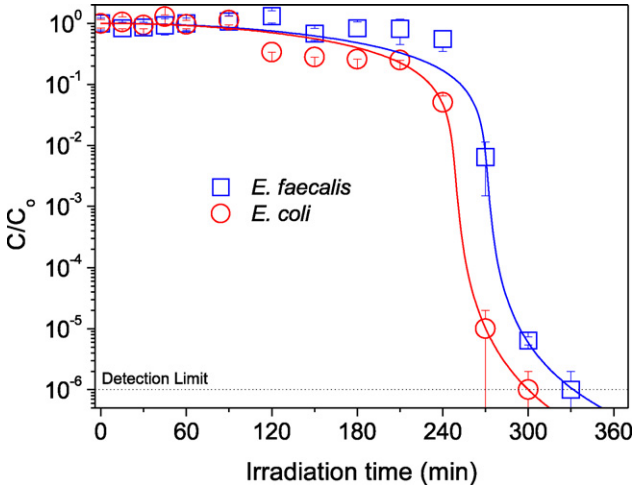

Fig. 10. Photocatalytic inactivation of a 50/50 mixture of *E. coli* and *E. faecalis* in a WTP simulated effluent using immobilized TiO_2 (TiO_2 concentration: 0.1 g L^{-1}; irradiation power: 2.8×10^{-6} Einstein s^{-1}; initial concentration of bacteria: 10^6 CFU mL^{-1}). Fitting lines were calculated using a kinetic model based on a series event disinfection mechanism [8]. Error bars calculated from eight independent bacteria counting measurements.

effluents) TiO_2 would be negatively charged, and then surrounded by a cations layer. Since *E. coli* is more negatively charged than Gram-positive bacteria in a range of pH from 4 to 9 [41], the *E. coli* attraction towards the catalyst might be more favored than that of *E. faecalis*.

3.3.1. Effect of irradiation power

Fig. 8 shows the influence of the irradiation power in the *E. coli* and *E. faecalis* photocatalytic inactivation, both in deionized water (figures) and WTP simulated effluent (inset). In all cases it is observed an increase in the disinfection efficiency as the irradiation power increases, obviously due to the higher amount of available photons to activate the TiO_2 catalyst, and therefore a higher production of hydroxyl radicals. Concerning the differences in the shape of the curves and the influence of the water composition, the results confirm the apparently different interaction existing between *E. faecalis* and *E. coli* and the immobilized TiO_2, attributed to differences in the charge and hydrophobicity of the cell wall. In any case, despite the significantly higher inactivation rate of *E. coli* cell at short irradiation times, the duration of the treatment to reach the bacterial detection limit is comparable for both microorganisms.

3.3.2. Effect of the initial concentration of bacteria

Experiments with increasing initial concentration of bacteria are shown in Fig. 9. The increase in the irradiation time required for the total inactivation of more concentrated bacterial suspensions is not as significant as the increase in the amount of bacteria, being all the curves very similar in terms of C/C_0 (not shown) for each type of bacteria and water. Consequently, as it happens for experiments TiO_2 in suspension, for equal irradiation conditions, the kinetics of the process is controlled by the concentration of bacteria.

Fig. 9 also confirms the reported differences in the shape of the inactivation curves of both microorganisms, with an initially faster inactivation of *E. coli* but a delayed *E. faecalis* inactivation that leads to comparable irradiation times to reach the bacterial detection limit, especially in deionized water. These results suggest again the different interaction with TiO_2 between Gram-positive and Gram-negative bacteria, independently on the experimental conditions.

3.3.3. Mixtures of E. coli and E. faecalis

Finally, experiments of photocatalytic inactivation of mixtures with similar initial concentrations of *E. coli* and *E. faecalis* in WTP simulated effluent were carried out to check the influence of these differences observed in the interaction of both types of bacteria with the immobilized TiO_2. In this case, both curves shown in Fig. 10 are quite similar showing a fast inactivation after a long initial delay, or in other words, the typical shape showed by *E. faecalis* curves. As it happens when using TiO_2 in suspension, although the plot suggest that *E. coli* seems to be slightly more sensitive to the treatment, these differences can not be considered as significant taking into account that both microorganisms have been quantified in selective culture media with slightly different selectivity.

Summarizing, despite the important differences found between *E. coli* and *E. faecalis* concerning the sensibility to osmotic stress, and its combination with mechanical and radiative stress, and also the different interaction bacteria-TiO_2 that makes the inactivation profiles significantly different especially in immobilized catalysts with low surface area, our results suggest that the impact on the irradiation treatment required for the total disinfection is quite low.

4. Conclusions

Non-photocatalytic inactivation experiments show that *E. coli* is more sensitive than *E. faecalis* when the osmotic stress coming from suspensions in deionized water was combined with other stress sources, such as stirring or UVA light. Despite the more complex external structure of Gram-negative bacteria, osmotic stress may induce a higher weakening of *E. coli* cell wall, enhancing the permeability to the oxidant species. However, the composition of water plays an important role, as no differences between both microorganisms were found in WTP simulated effluent, in which osmotic stress is significantly reduced.

Photocatalytic experiments with TiO_2 in suspension do not show any differences between the inactivation efficiency of *E. coli* and *E. faecalis*, both in deionized and WTP simulated effluent. Both microorganisms seem to follow the same inactivation mechanism, being similar the influence of operational variables such as catalyst concentration and irradiation power, responsible for the rate of hydroxyl radical generation. Moreover, differences in morphology and chemical composition, charge or hydrophobicity of the external cell structure between both types of bacteria do not seem to lead to significant differences in the microorganism-catalyst adhesion, showing similar inactivation profiles and comparable irradiation times required for the total inactivation below the detection limit. Consequently, *E. coli* photocatalytic inactivation results with TiO_2 in suspension can be extrapolated to the photocatalytic inactivation of other types of bacteria.

In contrast, some differences in *E. coli* and *E. faecalis* inactivation curves appeared when immobilized TiO_2 was used, especially in WTP simulated effluent, showing *E. faecalis* a much longer initial delay. In this case, the differences in morphology and in chemical composition of the external cell structure can lead to differences in electrostatic charge or hydrophobicity and consequently can also lead to differences in the bacteria-catalyst adhesion. This aspect is enhanced when TiO_2 is immobilized, since the available titania surface for bacteria-catalyst interaction is reduced in comparison with TiO_2 in suspension. However, despite these differences in the shape of the inactivation profiles, both types of microorganisms required similar irradiation times to achieve the complete inactivation, being this fact confirmed by experiments of inactivation of mixtures of *E. coli* and *E. faecalis*. Therefore, for real water disinfection applications, conclusions provided by experiments using *E. coli* as model microorganism could be also reasonably extrapolated to other types of bacteria consortia when using immobilized TiO_2, making possible the development of continuous water disinfection processes with purposes of drinking water supply or reuse of WTP effluents.

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References

[1] L.K. Wang, P.C. Yuan, Y.T. Hung, in: L.K. Wang, Y.T. Hung, N.K. Shammas (Eds.), Handbook of Environmental Engineering: Physicochemical Treatment Processes, Humana Press, Totowa, NJ, 2004, pp. 271–314.
[2] J.M. Herrmann, Catal. Today 53 (1999) 115–129.
[3] World Health Organization, Water, Sanitation and Hygiene Links to Health. Facts and Figures, 2004.
[4] J. Blanco-Gálvez, P. Fernández-Ibáñez, S. Malato-Rodríguez, J. Solar Energy Eng. 129 (2007) 4–15.
[5] C. McCullagh, J.M.C. Robertson, D.W. Bahnemann, P.K.J. Robertson, Res. Chem. Intermed. 33 (2007) 359–375.
[6] J.A. Herrera Melián, J.M. Doña Rodríguez, A. Viera Suárez, E. Tello Rendón, C. Valdés do Campo, J. Araña, J. Pérez Peña, Chemosphere 41 (2000) 323–327.
[7] A.G. Rincón, C. Pulgarín, Appl. Catal. B: Environ. 44 (2003) 263–284.
[8] J. Marugán, R. van Grieken, C. Sordo, C. Cruz, Appl. Catal. B: Environ. 82 (2008) 27–36.
[9] R. van Grieken, J. Marugán, C. Sordo, C. Pablos, Catal. Today 144 (2009) 48–54.
[10] T.P. Cushnie, P.K.J. Robertson, S. Officer, P.M. Pollard, C. McCullagh, J.M.C. Robertson, Chemosphere 74 (2009) 1374–1378.
[11] S. Malato, J. Blanco, D.C. Alarcón, M.I. Maldonado, P. Fernández-Ibáñez, W. Gernjak, Catal. Today 122 (2007) 137–149.
[12] M. Cho, H. Chung, W. Choi, J. Yoon, Water Res. 38 (2004) 1069–1077.
[13] J.A. Ibáñez, M.I. Litter, R.A. Pizarro, J. Photochem. Photobiol. A: Chem. 157 (2003) 81–85.
[14] Z. Huang, P.-C. Maness, D.M. Blake, E.J. Wolfrum, J. Photochem. Photobiol. A: Chem. 130 (2000) 163–170.
[15] M.T. Madigan, J.M. Martinko, J. Parker, Brock Biology of Microorganisms, 10th edition, Pearson Prentice Hall, 2003.
[16] A. Pal, S.O. Pehkonen, L.E. Yu, M.B.V. Ray, J. Photochem. Photobiol. A: Chem. 186 (2007) 335–341.
[17] M. Kositzi, I. Poulios, S. Malato, J. Cáceres, A. Campos, Water Res. 38 (2004) 1147–1154.
[18] C. Sichel, J. Blanco, S. Malato, P. Fernández-Ibañez, J. Photochem. Photobiol. A: Chem. 189 (2007) 239–246.
[19] J.M.C. Robertson, P.K.J. Robertson, L.A. Lawton, J. Photochem. Photobiol. A: Chem. 175 (2005) 51–56.
[20] C.-J. Chung, H.-I. Lin, C.-M. Chou, P.-Y. Hsieh, C.-H. Hsiao, Z.-Y. Shi, J.-L. He, Surf. Coat. Technol. 203 (2009) 1081–1085.
[21] A.G. Rincón, C. Pulgarín, Appl. Catal. B: Environ. 49 (2004) 99–112.
[22] A.I. Gomes, J. Santos, V.J.P. Vilar, R.A.R. Boaventura, Appl. Catal. B: Environ. 88 (2009) 283–291.
[23] G. Fu, P.S. Vary, C.-T. Lin, J. Phys. Chem. B 109 (2005) 8889–8898.
[24] K. Page, R.G. Palgrave, I.P. Parkin, M. Wilson, S.L.P. Savin, A.V. Chadwick, J. Mater. Chem. 17 (2007) 95–104.
[25] Y. Koizumi, M. Taya, Biotechnol. Lett. 24 (2002) 459–462.
[26] G. Gogniat, M. Thyssen, M. Denis, C. Pulgarín, S. Dukan, Microbiol. Lett. 258 (2006) 18–24.
[27] F. Manjón, L. Villén, D. García-Fresnadillo, G. Orellana, Environ. Sci. Technol. 42 (2008) 301–307.
[28] P. Wu, R. Xie, J.A. Imlay, J.K. Shang, Appl. Catal. B: Environ. 88 (2009) 576–581.
[29] K. Sunada, T. Watanabe, K. Hashimoto, J. Photochem. Photobiol. A: Chem. 156 (2003) 227–233.
[30] V.A. Nadtochenko, A.G. Rincón, S.E. Stanca, J. Kiwi, J. Photochem. Photobiol. A: Chem. 169 (2005) 131–137.
[31] K.P. Kühn, I.F. Chaberny, K. Massholder, M. Stickler, V.W. Benz, H.-G. Sonntag, L. Erdinger, Chemosphere 53 (2003) 71–77.
[32] O. Seven, B. Dindar, S. Aydemir, D. Metin, M.A. Ozinel, S. Icli, J. Photochem. Photobiol. A: Chem. 165 (2004) 103–107.
[33] Y. Lan, C. Hu, X. Hu, J. Qu, Appl. Catal. B: Environ. 73 (2007) 354–360.
[34] E. Bae, J.W. Lee, B.H. Hwang, J. Yeo, J. Yoon, H.J. Cha, W. Choi, Chemosphere 72 (2008) 174–181.
[35] E.V. Skorb, L.I. Antonouskaya, N.A. Belyasova, D.G. Shchukin, H. Möhwald, D.V. Sviridov, Appl. Catal. B: Environ. 84 (2008) 94–99.
[36] L. Shaomin, G. Gaoli, X. Bihua, G. Wenqi, M. Guangjum, J. Wuhan Univ. Technol. 24 (2009) 557–561.
[37] H.L. Liu, T.C.-K. Yang, Proc. Biochem. 39 (2003) 475–481.
[38] A.-G. Rincón, C. Pulgarín, Catal. Today 101 (2005) 331–344.
[39] A.I. Gomes, V.J.P. Vilar, R.A.R. Boaventura, Catal. Today 144 (2009) 55–61.
[40] B. Li, B. Logan, Colloids Surf. B 36 (2004) 81–90.
[41] C. Hu, X. Hu, J. Guo, J. Qu, J. Am. Chem. Soc. 40 (2006) 5508–5513.
[42] V. Cohen-Yaniv, N. Narkis, R. Armon, Water Sci. Technol. 58 (1) (2008) 247–252.
[43] L. Villén, F. Manjón, D. García-Fresnadillo, G. Orellana, Appl. Catal. B: Environ. 69 (2006) 1–9.
[44] T.N. Demidova, R. Hamblin, Antimicrob. Agents Ch. 49 (2005) 2329–2335.
[45] L.W. Sinton, C.H. Hall, P.A. Lynch, R.J. Davies-Colley, Appl. Environ. Microbiol. 68 (2002) 1122–1131.
[46] J. Marugán, R. van Grieken, C. Pablos, C. Sordo, Water Res. 44 (2010) 789–796.
[47] X.Z. Li, M. Chang, H. Chua, Water Sci. Technol. 33 (1996) 111–118.
[48] N. Huang, Z. Xiao, D. Huang, C. Yuan, Supramol. Sci. 5 (1998) 559–564.
[49] A.K. Benabbou, Z. Derriche, C. Felix, P. Lejeune, C. Guillard, Appl. Catal. B: Environ. 76 (2007) 257–263.
[50] F. Chen, X. Yang, F. Xu, Q. Wu, Y. Zhang, Environ. Sci. Technol. 43 (2009) 1180–1184.
[51] P.S.M. Dunlop, J.A. Byrne, N. Manga, B.R. Eggins, J. Photochem. Photobiol. A: Chem. 148 (2002) 355–363.
[52] D. Gumy, C. Morais, P. Bowen, C. Pulgarín, S. Giraldo, R. Hajdu, J. Kiwi, Appl. Catal. B: Environ. 63 (2006) 76–84.
[53] C. Sichel, J. Tello, M. de Cara, P. Fernández-Ibáñez, Catal. Today 129 (2007) 152–160.
[54] L. Vincze, T.J. Kemp, J. Photochem. Photobiol. A: Chem. 87 (1995) 257–260.
[55] M. Kerr, M. Fitzgerald, J.J. Sheridan, D.A. McDowll, I.S. Blair, J. Appl. Microbiol. 87 (1999) 833–841.
[56] T.-H. Bui, C. Felix, S. Pigeot-Remy, J.-M. Herrmann, P. Lejeune, C. Guillard, J. Adv. Oxid. Technol. 11 (2008) 510–518.
[57] P. Fernández Ibáñez, in: M.A. Blesa, B. Sánchez (Eds.), Eliminación de contaminantes por fotocatálisis heterogénea, Ciemat, Madrid, 2004, pp. 259–276.
[58] J.A. Ibañez, M.I. Litter, R.A. Pizarro, J. Photochem. Photobiol. A: Chem. 157 (2003) 81–85.
[59] C. Sichel, M. de Cara, J. Tello, J. Blanco, P. Fernández-Ibáñez, Appl. Catal. B: Environ. 74 (2007) 152–160.